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Zoological Research

Electrophoretic Analysis of Isozymes and Discussion about Species Differentiation in Three Species of Genus *Gymnocypris*

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Abstract: By using the method of electrophoresis, three isozymes (lactate dehydrogenase, malate dehydrogenase and esterase) of three species of genus *Gymnocypris* were described and analyzed from North Tibet in this paper. The results showed that all three isozymes presented interspecific difference and distinct differentiation among individuals in the same population, and there was no electrophorectic difference between males and females. Analysis of relationships among three naked carps indicated a high degree of similarity between *G. selincuoensis* and *G. cuoensis*, whereas low degree between *G. selincuoensis* and *G. namensis*. Furthermore, three isozymes presented expression of null alleles, and the duplicate genes of LDH-A^2, LDH-B^2, s-MDH-A^2 and m-MDH-B^2 also expressed in some individuals. Compared to other tetraploid fishes, three naked carps retained more functional duplicate genes and null alleles. This suggests fishes of genus *Gymnocypris* are at the early stage of evolution after polyploidization than that of fishes of Catostomidae, it directly related to the later time of schizothoracine fishes originate as well as severe environment.

Key words: Naked carps (*Gymnocypris*); North Tibet; Isozyme electrophoresis; Duplicate gene; Null allele; Species differentiation

The technique of isozyme electrophoresis is widely used in studying genetic expression and regulation in ontogeny (Wu & Wang, 1987, 1992; Wang & Liu, 1994), investigating evolution and estimating the relationship among species (Stoneking *et al*., 1981a), and analyzing genetic structures of population and genotypic biodiversity (Li *et al*., 1991; Wu & Wang, 1991). However, there is not any isozyme work on fishes in the Tibetan Plateau before. Schizothoracine fishes are a special group with its origination and evolution closely relating to the uplift of Tibetan Plateau (Qinghai-Xizang Plateau). It constitutes the main part of Tibetan fishes with the species of the genus *Triplophysa* of the subfamily Nemacheilinae. Fishes of the Tibetan Plateau intensively distribute in the Plateau and its adjacent areas (Cao *et al*., 1981; Wu & Wu, 1992; Chen & Chen, 1998). Genus *Gymnocypris* belongs to the subfamily Schizothoracinae of Cyprinidae, with total 12 species and subspecies (Chen & Cao, 2000), is one of the highly specialized groups in the schizothoracine fishes.

In this study, we examined lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and esterase (EST) of three species of genus *Gymnocypris*, which only distributed in the Namucuo Lake, Cuoe Lake, Selincuo Lake and its tributary Zhajiazangbu River and Zhagenzangbu River in North Tibet respectively. On the basis of described characters of isozyme expression and differentiation, we have discussed the species differentiation of naked carps.

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1 Materials and Methods

1.1 Study animals

From May to July in 1998, we collected respectively *G. namensis*, *G. selincuoensis* and *G. cuoensis* from the Namucuo Lake (4 718 m asl.), Selincuo Lake (4 530 m asl.) and Cuoe Lake (4 562 m asl.). All fish specimens were weighed (± 1 g) and measured standard length (± 1 mm) and were kept alive until tissue was collected. Tissue samples were taken from liver, heart, white muscle, kidney and gonad and were preserved in liquid nitrogen. The stages of gonadal development were determined according to method of Мейен (1939). Finally, 17 specimens of *G. selincuoensis*, 10 specimens of *G. Cuoensis* and 8 specimens of *G. namensis were* examined with PAGE.

1.2 Electrophoresis procedures

Tissues samples preserved in liquid nitrogen were extracted. The samples were weighed 0.2 g and mixed in glass homogenizers respectively with 1 mL 0.01 mol/L cold $K_2HPO_4-KH_2PO_4$ buffer (pH 7.00), and homogenized in ice-bath, and centrifuged at 12 000 r/min for 20 min at 4℃. The supernatants were used for electrophoresis.

Vertical polyacrylamide gel electrophoresis was employed to separate isozymes for the analysis. Stacking gel containing was 4% arylamide and 20% bis-arylamide. The separation gel containing was 8.0% arylamide and 3% bis-arylamide. The electrode buffer was 0.005 mol/L Tris - 0.3 mol/L Glycine, pH 8.30. 30 μL supernatant sample was put at top gel. The electrophoresis was carried out with a current of 1.5 mA/cm at 4℃, LDH, MDH and EST for 4 hr.

1.3 Staining procedures

Histochemical staining procedures for LDH, MDH and EST followed Shaw & Prasad (1970). The isozyme electrophoretograms were analyzed with number of bands, relative mobility and staining intensity. Allelic terminology was adopted by Avise & Ayala (1976). One allele, usually the most common in *Gymnocypris*, was designated as 100. The number designated to other alleles is the distance moved from the origin towards the anode expressed as a percentage of the distance move by allele 100. Null allele was marked 0 with superscript and duplicate gene was marked 2 with superscript.

1.4 Analysis of allozyme data

Chi-square tests were used to determine weather observed genotypic ratios deviated significantly from Hardy-Weinberg expectation. The average heterozygosity ($\overline{H}$) of a population was estimated from allele frequency data, using the formula:

$$\overline{H} = 1 - \sum_{i=1}^{n} x_i^2$$

Where x_i is the frequency of the ith allele at a locus, with n alleles, and averaging values over all loci. Genetic relationships among three species were assessed using Rogers' (1972) index of genetic similarity (S) and Nei's (1972) genetic distance (D), which were expressed as follows:

$$S = 1 - [1/2\sum_{i=1}^{n}(P_{ix} - P_{iy})^2]^{1/2}$$

Where P_{ix} is frequency of allele i in population x, P_{iy} is frequency of allele i in population y, and n is number of alleles at the locus. As above, the genetic distance $D = -\ln S$.

2 Results

2.1 Lactate dehydrogenase (EC1.1.1.27)

The result showed that the bands were the darkest near cathode in liver and white muscle, so the migrating of A_4 subunit in the main band was the slowest with genotype a_4. The bands were the darkest near anode in heart, so B_4 subunit in the main band migrated fastest with genotype b_4. Therefore, the main five bands of LDH electrophoretograms were respectively B_4, B_3A_1, B_2A_2, B_1A_3 and A_4 subunit from anode to cathode (Fig.1).

From LDH isozyme electrophoretograms, we have observed that expression quantity of isozyme in LDH-A and LDH-B loci presented admittedly difference in liver of three naked carps. Activity or produce of LDH-B subunit distinctly got down and even had A_4 subunit.

Except the difference of five main bands, there were sub-bands, which were less intense than that of the main bands in LDH electrophoretograms of three

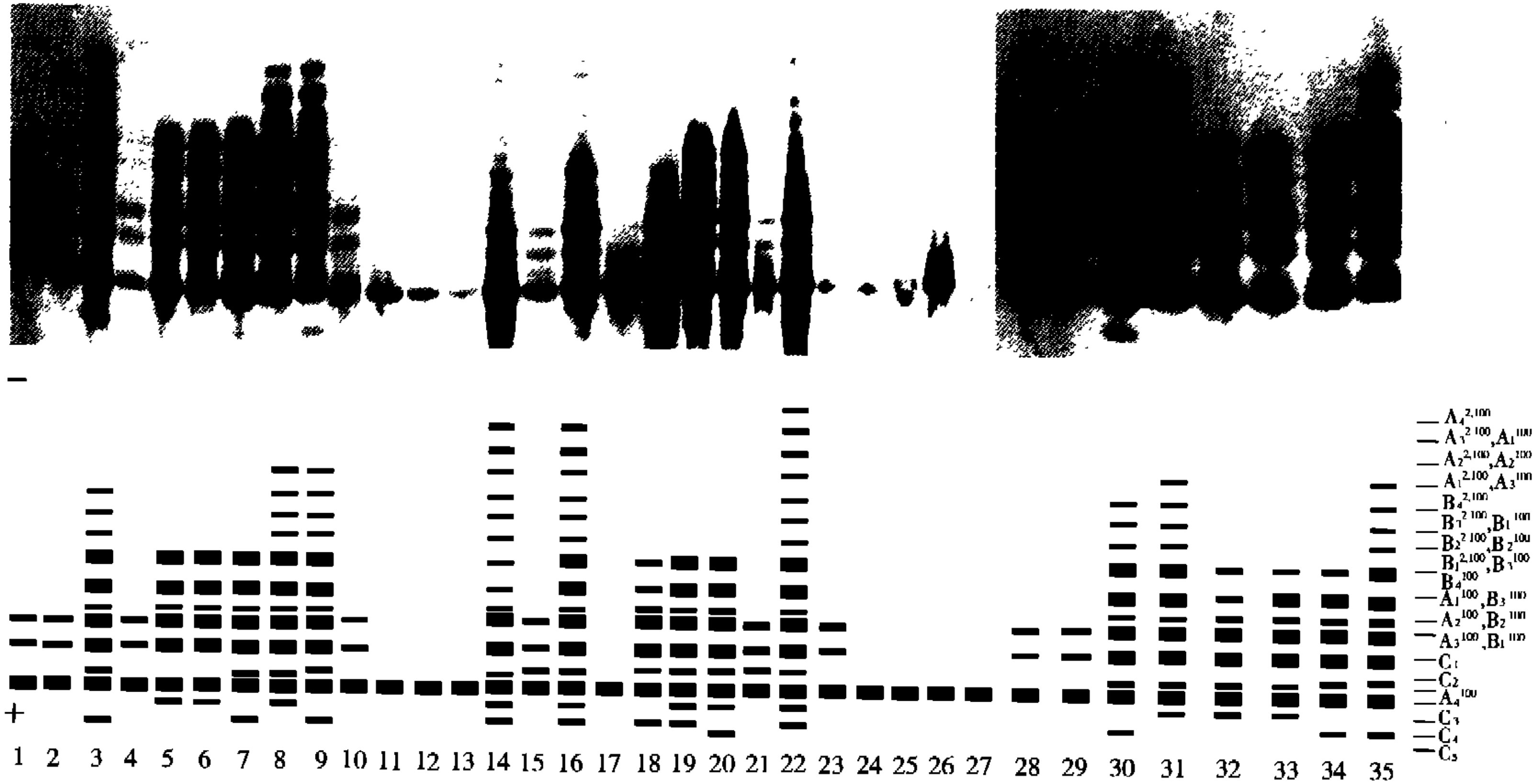


Fig.1 LDH isozyme electrophoretograms of naked carps from North Tibet

1 – 10: *G. cuoensis*; 11 – 27: *G. selincuoensis*; 28 – 35: *G. namensis*; 1,4,6,7,8,10,11,12,13,16,19,21,22,25,28,30,31,33 and 34: female; 2,3,5,9,14,15,17,18,20,23,24,26,27,29,32 and 35: male; 1,2,4,10,15,21,23,28 and 29: null allele of LDH-B expressed; 3,8,9,30,31 and 35: duplicate gene of LDH-B^2 expressed; 5,6,7,19,20,32,33 and 34: null allele of LDH-B^2 expressed; 11,12,13,24,25,26 and 27: null alleles of LDH-A^2, B^2 expressed; 14: duplicate genes of LDH-A^2, B^2 and null allele of LDH-B expressed; 16 and 22: duplicate genes of LDH-A^2, B^2 expressed; 22 near to anode sub-bands (C_5) mobility present different.

naked carps, and the sub-bands mobility appeared distinct differentiation among individuals in same population.

There were not significant difference between males and females, at stages of gonadal development and among the fish with different ages in zymogram of all three naked carps.

The frequencies of three polymorphic loci LHD-B, LHD-A^2 and LHD-B^2 in different populations of three naked carps were given in Table 1. All alleles in *G. namensis*, no genotypic ratios deviated significantly from Hardy-Weinberg expectation ($P>0.05$). Nevertheless, all alleles in *G. selincuoensisi* deviated significantly from Hardy-Weinberg expectation ($P<0.01$). Among three naked carps, *G. selincuoensis* and *G. cuoensis* exhibited a high genetic variability. Two species displayed higher frequency of null allele LDH-A. Duplicate gene LDH-B^2 was expressed in *G. selincuoensis* and *G. cuoensis* but not in *G. namensis* (Table 1). Furthermore, *G. selincuoensis* displayed a distinct polymorphisms at locus C. With exception of other 4 bands that were relative to locus C, there was a unique C_5 band in electrophoretograms (Fig.1), which was absent in *G. cuoensis* and *G. namensis*.

2.2 Malate dehydrogenase (EC1.1.1.37)

MDH isozyme is a dimer. There are two types of supernatant (s) and mitochondrial (m) MDH in *G. selincuoensis* and *G. cuoensis*, but all individuals of *G. namensis* only displayed the supernatant type MDH and showed consistency highly. The zymograms presented distinct differentiation among individuals in *G. selincuoensis* and in *G. cuoensis*. The subunits of s-MDH-A and s-MDH-B even formed a heterodimer. Electrophoretic chromogen of m-MDH isozyme was fairly unstable and there were many sub-bands in two types of MDH isozyme. These would be the result of the expressions of the two duplicate genes of s-MDH-A^2 and m-MDH-B^2. In some individuals of *G. selincuoensis* and *G. cuoensis*, locus s-MDH-A or m-MDH-B displayed null allele and exhibited polymorphism. It is sure that activity of s-MDH and m-MDH was quite significantly different among individuals of the two species. However, there was no expression of null allele at locus s-MDH-A in *G. namensis* (Fig.2). The frequencies of two polymorphic loci s-MDH-A and m-MDH-B of three naked carps were given in Table 1. All alleles in *G. selincuoensis* and *G. cuoensis*, no genotypic ratios deviated significantly from Hardy-Weinberg expectation ($P>0.05$).

2.3 Esterase (EC31.1.1.1)

In the liver of these naked carps, EST isozyme could be partitioned into seven main bands and four to seven sub-bands which encoded at least seven loci (Fig.3). Among them, each of the six loci (Est-1, Est-2, Est-10, Est-11, Est-13 and Est-14) had polymorphism and a null allele. The frequencies of 6 polymorphic loci in populations of three naked carps were given in Table 1. All alleles in *G. namensis*, no genotypic ratios deviated significantly from Hardy-Weinberg expectation ($P>0.05$). However, both *G. selincuoensis* and *G. cuoensis*, the frequencies of alleles except Est-10 and Est-14 deviated significantly from Hardy-Weinberg expectation. In populations of *G. selincuoensis* and *G. cuoensis*, Loci Est-1, Est-2 and Est-14 were only observed two homozygotes (Est-$1^{100/100}$, Est-$1^{0/0}$; Est-$2^{100/100}$, Est-$2^{0/0}$ and Est-$14^{100/100}$, Est-$14^{0/0}$). However, there were three genotypes at locus Est-14 of *G. namensis*, two homozygotes (Est-$14^{100/100}$, Est-$14^{0/0}$) and one heterozygote (Est-$14^{100/0}$). Loci Est-11 and Est-13 were exhibited three genotypes, two homozygotes (Est-$11^{100/100}$, Est-$11^{0/0}$ and Est-$13^{100/100}$, Est-$13^{0/0}$) and one heterozygote (Est-$11^{100/0}$ and Est-$13^{100/0}$) respectively. Locus Est-11 was observed four genotypes, three homozygotes (Est-$11^{100/100}$, Est-11^{90}, Est-$11^{0/0}$) and one heterozygote (Est-$11^{100/0}$) in *G. namensis*. Locus Est-12^{100} was fixed in all individuals and every population of three naked carps, and which suggested that locus Est-12 was monomorphic in three naked carps.

2.4 Levels of genic variability and genetic similarity

The percentage of polymorphic loci and average heterozygosity ($\overline{H}$) for populations of three naked carps was presented in Table 2. *G. selincuoensis* and *G. cuoensis* had a higher proportion of polymorphic loci

Table 1　The allele frequencies at polymorphic loci and chi-squared test for three naked carps from North Tibet

Allozyme locus	Genotype	*Gymnocypris selincuoensis* Observed value	Predicted value	χ^2	P	Frequency of allele		*Gymnocypris cuoensis* Observed value	Predicted value	χ^2	P	Frequency of allele		*Gymnocypris namensis* Observed value	Predicted value	χ^2	P	Frequency of allele		
LDH-B	100/100	6.00	2.25			100	0	6.00	3.60			100	0	6.00	6.00			100		0
	100/0	0.00	7.50	28.13	<0.01	0.38	0.62	0.00	4.80	11.52	<0.01	0.60	0.40	0.00	1.50	1.50	0.25~0.75	0.75		0.25
	0/0	10.00	6.25					4.00	1.60					2.00	0.50					
LDH-A^2	100/100	3.00	0.56			100	0	0.00	0.00			100	0	0.00	0.00			100		0
	100/0	0.00	4.88	11.91	<0.01	0.19	0.81	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00		1.00
	0/0	13.00	10.56					10.00	10.00					8.00	8.00					
LDH-B^2	100/100	3.00	0.56			100	0	3.00	0.90			100	0	3.00	3.00			100		0
	100/0	0.00	4.88	11.91	<0.01	0.19	0.81	0.00	4.20	8.82	0.02~0.05	0.30	0.70	0.00	1.87	2.33	0.25~0.75	0.38		0.62
	0/0	13.00	10.56					7.00	4.90					5.00	3.13					
m-MDH-B	100/100	5.00	5.28			100	0	4	2.87			100	0	0.00	0.00			100		0
	100/0	3.00	2.46	0.15	>0.99	0.81	0.19	1	3.22	2.42	0.25~0.55	0.64	0.36	0.00	0.00	0.00	1.00	0.00		0.00
	0/0	0.00	0.26					2	0.91					0.00	0.00					
s-MDH-A	100/100	7	6.13			100	0	6	2.28			100	0	10.00	10.00			100		0
	100/0	0	1.74	1.51	0.75~0.80	0.88	0.12	0	3.43	8.54	0.02~0.05	0.86	0.14	0.00	0.00	0.00	1.00	1.00		0.00
	0/0	1	0.13					1	1.29					0.00	0.00					
EST-1	100/100	7.00	4.90			100	0	5.00	2.50			100	0	3.00	2.5			100		0
	100/0	0.00	4.20	8.82	0.02~0.05	0.70	0.30	0.00	5.00	12.50	<0.01	0.50	0.50	4.00	5.00	0.50	0.90~0.95	0.50		0.50
	0/0	3.00	0.90					5.00	2.50					3.00	2.50					
EST-2	100/100	7.00	4.90			100	0	5.00	2.50			100	0	3.00	2.50			100		0
	100/0	0.00	4.20	8.82	0.02~0.05	0.70	0.30	0.00	5.00	12.50	<0.01	0.50	0.50	4.00	5.00	0.50	0.90~0.95	0.50		0.50
	0/0	3.00	0.90					5.00	2.50					3.00	2.50					
EST-10	100/100	0.00	0.10			100	0	0.00	0.23			100	0	3.00	1.60			100		0
	100/0	2.00	1.80	0.02	0.97~0.99	0.10	0.90	3.00	2.55	0.10	>0.99	0.15	0.85	2.00	4.80	3.92	0.25~0.45	0.40		0.60
	0/0	8.00	8.10					7.00	7.22					5.00	3.60					
EST-11	100/100	2.00	3.60			100	0	1.00	3.03			100	0	0.00	0.90			100	90	0
	100/0	8.00	4.80	5.12	0.01~0.05	0.60	0.40	9.00	4.95	8.20	0.03~0.05	0.55	0.45	6.00	3.00	3.01	0.35~0.65	0.30	0.20	0.50
	90/90	0.00	0.00					0	0					2.00	0.40					
	0/0	0.00	1.60					0.00	2.02					2.00	2.5					
EST-13	100/100	4.00	1.60			100	0	3.00	3.02			100	0	4.00	3.03			100		0
	100/0	0.00	4.80	11.52	<0.01	0.40	0.60	3.00	4.95	2.56	0.25~0.55	0.45	0.55	3.00	4.95	1.90	0.55~0.75	0.55		0.45
	0/0	6.00	3.60					4.00	2.03					3.00	2.02					
EST-14	100/100	1.00	0.10			100	0	0.00	0.00			100	0	1.00	0.23			100		0
	100/0	0.00	1.80	1.62	0.55~0.75	0.10	0.90	0.00	0.00	0.00	1.00	0.00	1.00	1.00	2.55	1.20	0.75~0.90	0.15		0.85
	0/0	9.00	8.10					10.00	10.00					8.00	7.22					

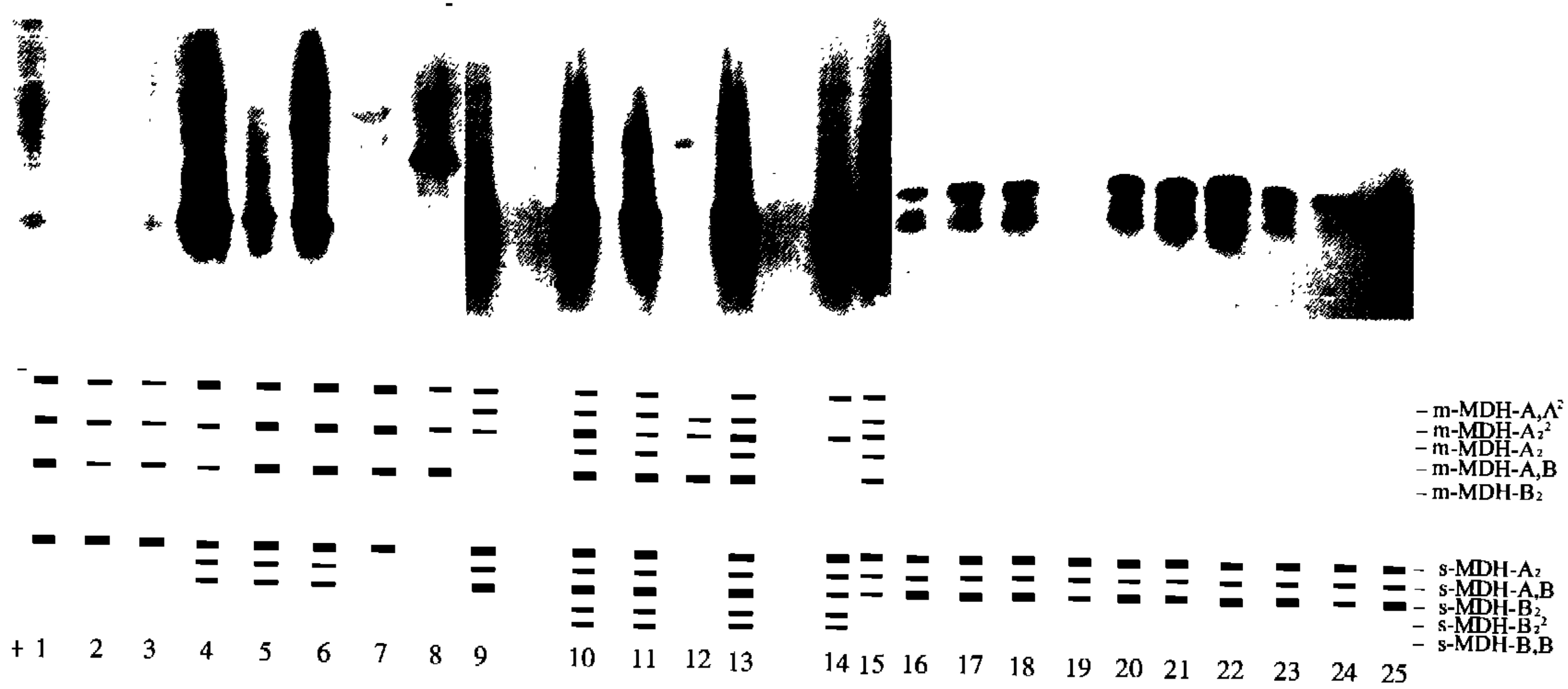
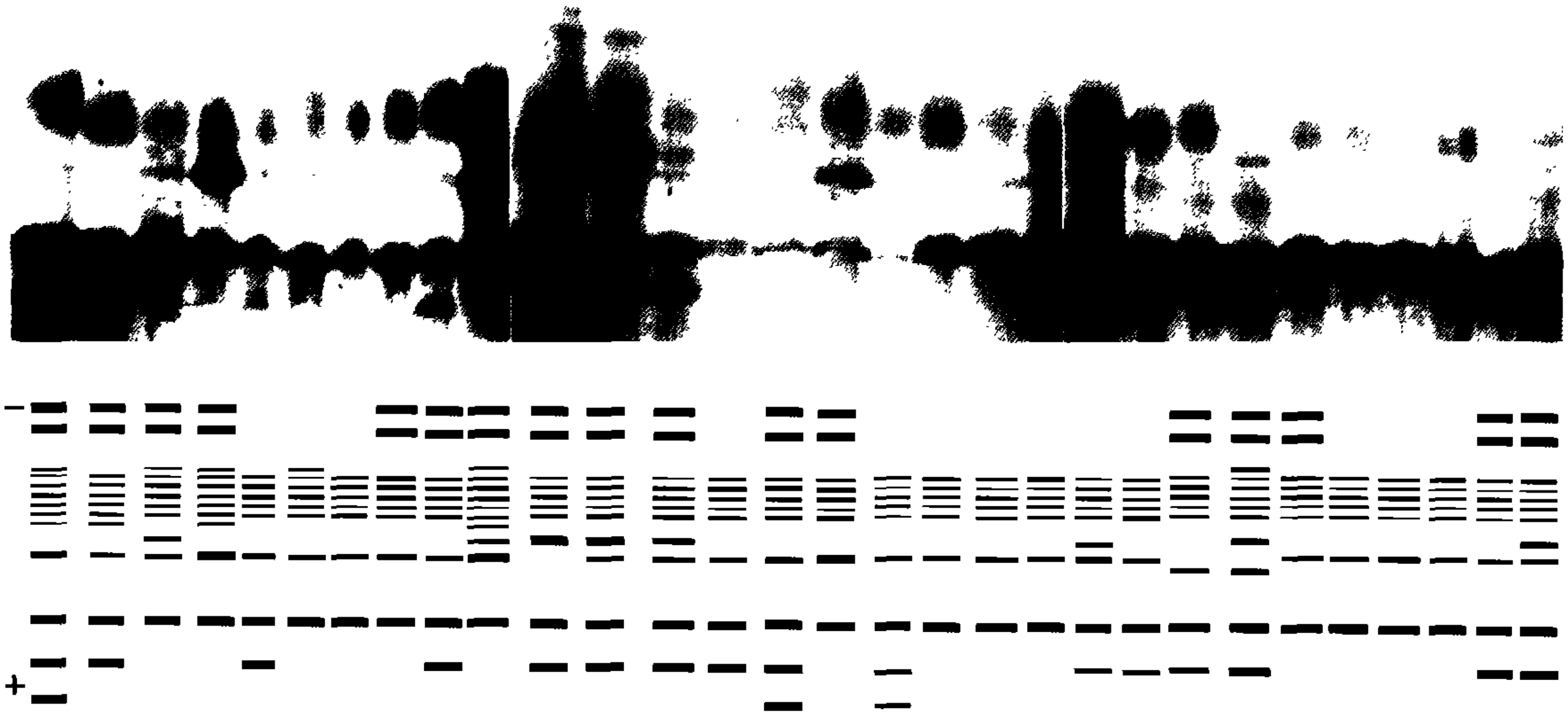


Fig.2 MDH isozyme electrophoretograms of naked carps from North Tibet

1-8: *G. selincuoensis*; 9-15: *G. cuoensis*; 16-25: *G. namensis*; 1,2,5,8,9,12,13,15,16,18,19,21,22,24 and 25: female; 3,4,6,7,10,11,14,17,20 and 23: male; 8,11 and 15: null alleles of m-MDH-A,A^2 expressed; m-MDH' activity or expressing quantity of 7,8, and 12 surpassed s-MDH's; 2,9,11 and 14: null alleles of m-MDH-A,B expressed; 16-25: only s-MDH expressed.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Est-1	100/100	100/100	100/100	100/100	0/0	0/0	0/0	100/100	100/100	100/100	100/100	100/100	100/100	0/0	100/100
Est-2	100/100	100/100	100/100	100/100	0/0	0/0	0/0	100/100	100/100	100/100	100/100	100/100	100/100	0/0	100/100
Est-3 -9															
Est-10	0/0	0/0	100/0	0/0	0/0	0/0	0/0	0/0	0/0	100/0	100/100	100/100	100/0	0/0	100/0
Est-11	100/0	100/0	100/0	100/100	100/0	100/0	100/0	100/0	100/0	100/100	0/0	100/0	100/0	100/0	100/0
Est-12	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Est-13	100/100	100/100	0/0	0/0	100/100	0/0	0/0	0/0	100/100	0/0	100/100	100/100	100/100	100/100	100/100
Est-14	100/100	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	100/100

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Est-1	100/100	0/0	0/0	0/0	0/0	0/0	0/0	100/100	100/100	100/100	0/0	0/0	0/0	100/100	100/100
Est-2	100/100	0/0	0/0	0/0	0/0	0/0	0/0	100/100	100/100	100/100	0/0	0/0	0/0	100/100	100/100
Est-3 -9															
Est-10	0/0	0/0	0/0	0/0	0/0	100/0	0/0	0/0	100/0	0/0	0/0	0/0	0/0	0/0	100/0
Est-11	100/100	100/0	100/0	100/0	100/0	100/0	100/0	90	90	100/0	100/0	100/0	100/0	100/0	100/0
Est-12	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Est-13	0/0	100/0	0/0	0/0	0/0	100/0	100/0	100/0	100/100	0/0	0/0	0/0	0/0	100/100	100/100
Est-14	0/0	100/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Fig.3 EST isozyme electrophoretograms of naked carps from North Tibet

1 – 10: *G. selincuoensis*; 11 – 20: *G. cuoensis*; 21 – 30: *G. namensis*; 1,2,3,6,9,11,14,16,17,18,20,21,23,24,26,27,29 and 30: female; 4,5,7,8,10,12,13,15,19,22,25 and 28: male.

and mean heterozygosity than *G*. *namensis*.

Table 3 presented a matrix of genetic similarity (S) and genetic distance (D) between all species were examined. Interspecific comparisons indicated a high degree of similarity between *G*. *selincuoensis* and *G*. *cuoensis*. While there were more differentiation between *G*. *namensis* and *G*. *selincuoensis* or *G*. *cuoensis*.

Table 2 Average heterozygosity ($\bar{H}$) and proportion of polymorphic loci (P) of three naked carps from North Tibet

	G. selincuoensis	*G. cuoensis*	*G. namensis*
Average heterozygosity ($\bar{H}$)	0.25	0.26	0.24
Proportion of polymorphic loci (P)	0.73	0.60	0.53

Table 3 Genetic relationships among three naked carps from North Tibet

	G. selincuoensis	*G. cuoensis*	*G. namensis*
G. selincuoensis	—	0.90	0.69
G. cuoensis	0.11	—	0.75
G. namensis	0.37	0.29	—

genetic distance (D) was given below the diagonal, and genetic similarity (S) was given above.

3 Discussion

As in mammal, there is probably a third LDH locus C (Xue, 1992) in most fishes of teleosts and its function is polarized into 2 classes: tendency to anode which is predominant in retina or tendency to cathode which is the main in liver. However, it never presents in both two tissues (Markert *et al*., 1975; Whitt *et al*., 1975; Xue, 1992). This study was used by vertical polyacrylamide gel electrophoresis, so there is impossible C_4 subunit which is LDH-C locus expressing product in liver. And C subunit forms not only homopolymer C_4, but also heteropolymer with A or B subunit, so near cathode sub-bands may be heteropolymer with A subunit or B subunit (Kirpichnikov, 1981). But its mobility was distinct differentiation and this indicated locus C and presented several alleles.

3.1 Expression of duplicate gene and its phylogenetic meaning

From the isozyme electrophoregrams above, it is obvious that there were a higher percent of duplicate genes and null alleles expression in three naked carps than those of other carps and minnows, and the phenomenon revealed that their population and even individual hold a distinct differentiation.

Like other eukaryotes, the increasing of cellular DNA content in fish has been an important process in their evolution (Ferris & Whitt, 1977a; Buth, 1979). At molecular level, Not all of duplicate genes would be always expressed (Buth, 1979; Crabtree & Buth, 1981). Along with gene reduplicating, many of the redundant copies would be silenced early, those remaining expressed are then available to diverge in structure and acquire new function. Ferris & Whitt (1977a) have found that the species of the primitive subfamilies (Cycleptinae and Ictiobinae) in family Catostomidae have remained more functionally duplicate genes than these of the advanced subfamilies (Catostominae), and considered that relatively primitive (pleimorphy) species have more functionally duplicate gene than morphologically divergent (apomorphy) species. This phenomenon has been examined in whole family Catostomidae and other tetraploid fishes (Ferris & Whitt, 1977b; Xiong & Xia, 1985; Luo & Wang, 1987). Therefore, expression or unexpression of the duplicate gene has the phylogenetic meaning. The existing schizothoracine fishes that have been studied were a tetraploid karyotype with 2n = 90 – 98 or even a hexaploid karyotype with 2n = 146 – 148, compared with 2n = 50 for most other species of Cypriniformes (Zan *et al*., 1985; Yu *et al*., 1989; Wu *et al*., 1999). Compared to other tetraploid fishes, all three naked carps retained a high expression level of duplicate gene. *Wu et al*. (1981) considered that family Cyprinidae was advanced than Catostomidae in phylogeny. In origin time, naked carp was the last one in these fish species. According to Ferris & Whitt (1977a, 1977b), three naked carps should lose more functional duplicate genes. Whereas three naked carps retained a high expression level of duplicate gene, which occupies 50.0% in loci of two examined dehydrogenases, and was somewhat equal to that of suckers (Ferris & Whitt, 1978). The high percent expression

of duplicate gene would be considered as a special case of naked carps to adapting severe environmental of the Plateau.

3.2 Null allele

The expression of null allele was found in all examined three isozymes in naked carps and its expression level was much higher than the other tetraploid fishes. It was commonly assumed that one of the duplicate loci was free to accumulate or would have been previously 'forbidden' mutations as long as other locus remains functional. Null allele may has drift to fixation shortly after polyploidization, especially in small population or species that niche restricted (Engel *et al*., 1973; Stoneking *et al*., 1981a, 1981b; Liu & Wang, 1997). To the polyploidy fish, null allele would not express in the procession from drift to fixation. Therefore, the status of s-MDH-A^2 existing null alleles and expression of duplicate genes LDH-A^2 and LDH-B^2 in the three naked carps should suggest that their populations were at the early stage after polyploidization.

3.3 Quite individual differentiation

Studies in other fish species have been indicated isozyme expression tissue and species-specific, but most individuals presented same genotype in intraspecies or intra-population (Li *et al*., 1991; Wu & Wang, 1991). Electrophoretic patterns of the three naked carps showed that it appeared a specific tissue distribution and difference not only among the populations, but also among individuals within the population. Among individuals of the limnic population in Selincuo Lake, expression of duplication genes LDH-A^2 and LDH-B^2 occupies 12.5% ($n = 16$), LDH-B^2 and LDH-B expressed as null allele respectively accounts for 18.7% and 62.5%. And that mobility of sub-bands near A_4 subunit in cathode had 5 types and represented distinct differentiation (Fig.1). Then MDH and EST show distinct differentiation too. Errors led by experiment condition could be obviated in this study because tissue samples of one population were taken for electrophoresis under same conditions and at the same time. Distinct differentiation among individuals of the three naked carps would be a special phenomenon.

However, the genetic similarity and distance between three naked carps showed that *G. selincuoensis* had more similar with *G. cuoensis* than *G. namensis*, this concurred with sequence of geological isolation among three lakes.

In summary, there are a relative high percent of duplicate gene and null expression as well as individuals differentiation in the three naked carps than other cyprinid fishes had reported (Buth & Burr, 1978; Wu & Wang, 1988), these may be direct correlative to latter derivation of schizothoracine fishes and the environmental variation of the Tibetan Plateau uplift (Cao *et al*., 1981; Chen, 1998). This process had carried through at different levels and was related to the stages of upheaval of the Tibetan Plateau. In temporal respect, the origin of schizothoracine fishes is comparatively later, most of duplicate genes and null alleles had been maintaining to express; that implicates that fishes of genus *Gymnocypris* were posterior to that of fishes of Catostomidae. In spatial respect, since the rapid uplift of the Tibetan Plateau, correspond conditions of the environment and climate have changed, original fishes were weeded out and this led to have a surplus of niche. Together with the continuously process of the uplift of the Tibetan Plateau, both cases of new existential conditions producing and old one disappearing exist all the time. Schizothoracine fishes presents a distinct differentiation at morphological, physiological, biochemical and even molecular levels (Chen & Chen, 2000), in favor of themselves to adapt incessantly changes of the plateau environment and to share every niche as quick as possible.

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藏北3种裸鲤同工酶的电泳分析及物种分化的探讨

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摘要：对藏北高原3种裸鲤的乳酸脱氢酶（LDH）、苹果酸脱氢酶（MDH）和脂酶（EST）进行电泳分析的结果表明，3种裸鲤酶谱均表现出种间的差别，而且在同一种群个体之间也存在着明显的分化，但无性别差异。3种裸鲤被检测的3种同工酶均有沉默基因表达的现象，重复基因 LDH - A^2、LDH - B^2、s - MDH - A^2 和 m - MDH - B^2 也在部分个体中表达。遗传距离分析表明，色林错裸鲤（*G. selincuoensis*）与错鄂裸鲤（*G. cuoensis*）之间较之于与纳木错裸鲤（*G. namensis*）有更近的亲缘关系。与其他四倍体鱼类相比，裸鲤鱼类同工酶在重复基因和沉默基因上都有较高的表达频率，这种情况说明裸鲤鱼类目前可能还处于多倍化后进化的早期过程并早于胭脂鱼类所处的相应时期，这与裂腹鱼类起源较晚以及青藏高原业已存在的恶劣环境条件直接相关。

关键词：裸鲤；藏北；同工酶电泳；重复基因；沉默基因；物种分化
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中国兽类学会成立20周年暨2000年度学术研讨会在济南召开

2000年11月27日至12月1日中国兽类学会成立20周年暨学术研讨会在泉城济南市山东大学召开。来自全国20多个省市的科研机构、大专院校、林业部门、农业部门、卫生防疫部门和动物园系统的120多位代表出席了这次会议。会议由第四届兽类学会副理事长胡锦矗教授主持，并由他宣读了张洁理事长的贺信；副理事长、《兽类学报》主编王祖望教授致开幕词并宣读了夏武平名誉理事长的贺信；中国动物学会理事长陈大元先生代表动物学会出席了本次会议并致贺辞；山东大学校长于修平教授作为东道主致欢迎辞；山东野生动物学会、山东省动物学会、山东大学生命科学学院等单位对本次会议在济南召开发来贺信表示祝贺。第四届学会秘书长冯祚建先生代表理事会做工作报告，对第四届理事会5年来的工作进行了回顾，并就学会的组织工作、学术活动和财务状况等向与会代表做了汇报。

本次大会共收到论文摘要86篇，学术研讨会分大会报告和分组报告。全体大会上王祖望教授做了"我国兽类学近20年的发展与展望"的报告，从学科发展、论文发表与专著出版、学术交流与国际合作以及人才培养和引进4个方面对我国兽类学的发展做了回顾；张知彬先生做了"种群时空动态的分子生物学机制"的报告，系统详细地介绍了目前该领域国外先进的研究方法及其重要的理论假说，对我国学者开展这方面的工作有重要的指导意义；胡锦矗教授的"大熊猫的现状与保护"报告了大熊猫的栖息地及大熊猫生存状况，提出了保护大熊猫的措施。分组报告分大型兽组和小型兽组，60多位代表在分组报告上展示了他们近年来的科研成果，论文涉及兽类学的各个研究领域。其中濒危动物保护及行为学的论文24篇，从不同层次、不同侧面讨论了濒危动物的现状，突出了人与自然的协调发展，并提出了相应的保护措施；分子生物学技术与方法在兽类学各学科的运用，也是本次学术研讨会的热点，共收到相关论文17篇，标志着我国兽类学的发展已进入了一个新的阶段；小型啮齿类的行为、种群生态、代谢生理的论文26篇，为我国农田鼠害、荒漠鼠害的生态综合治理提供了理论依据。与会专家相互交流，各抒己见，学术气氛十分浓厚。本届大会既看到我国一批中老科学家仍然坚持耕耘在兽类学研究的第一线，又看到一大批思想活跃，勇于探索和创新的青年学者已成为我国兽类学研究的中坚，其中有的正是充满活力的在读博士和硕士研究生，使我们看到了我国兽类学的发展和希望。

大会选举产生了新一届即第五届兽类学会理事会，新组成的理事会由56名成员组成，一批德高望重的老科学家继续担任学会理事，同时增选了一批年富力强的青年学者为理事。大家一致推举夏武平先生为名誉理事长，并经无记名投票选举王祖望教授为理事长，马逸清、张知彬、胡锦矗、赵新全、徐宏发教授为副理事长，魏辅文教授为秘书长。

大会在祥和、热烈的气氛中闭幕，取得了圆满的成功。

《兽类学报》编辑部